Lizard scales in an adaptive radiation: variation in scale number follows climatic and structural habitat diversity in Anolis lizards

Header

Scale variation in *Anolis* lizards

Title

Lizard scales in an adaptive radiation: variation in scale number follows climatic and structural habitat diversity in *Anolis* lizards

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Abstract

Lizard scales vary in size, shape and texture among and within species. The overall function of scales in squamates is attributed to protection against abrasion, solar radiation and water loss. We quantified scale number of *Anolis* lizards across a large sample of species (142 species) and examined whether this variation was related either to structural or climatic habitat diversity. We found that species in dry environments have fewer, larger scales than species in humid ones. This is consistent with the hypothesis that scales reduce evaporative water loss through the skin.
In addition, scale number varied among groups of ecomorphs and was correlated with aspects of the structural microhabitat (i.e. perch height and perch diameter). This was unexpected because ecomorph groups are based on morphological features related to locomotion in different structural microhabitats. Body scales are not likely to play an important role in locomotion in *Anolis* lizards. The observed variation may relate to other features of the ecomorph niche and more work is needed to understand the putative adaptive basis of these patterns.

Keywords: comparative methods – ecomorphology – precipitation

Introduction

The skin of lizards and snakes is covered by keratinized scales that limit water loss and offer protection from abrasion in the terrestrial realm and thus have contributed to the invasion of land by reptiles (Walker & Liem, 1994; Alibardi, 2003; Oufiero *et al.,* 2011). In snakes and limbless lizards scales are involved in locomotion in promoting movement through muscle activity, in passively engaging gliding and in minimizing backward movement (Gray, 1946, Kerfoot, 1969; 1970). The functional significance, however, of scale variation in other types of lizards—displayed as myriad shapes, sizes and textures— remains less well understood. Functional hypotheses often focus on temperature because ectothermic vertebrates are sensitive to fluctuations in their thermal environment (Huey *et al.*, 2009; Sinervo *et al.*, 2010). For instance, biophysical predictions suggest that larger scales displace more heat, and therefore larger-scaled animals will be found in areas where chronic overheating may be problematic (Soulé, 1966; Regal, 1975). In addition, squamates inhabit the full spectrum of hydric environments, from wet forests to some of the most arid areas on earth and rates of water loss across the skin (cutaneous water loss) vary drastically both intra- and inter-specifically in lizards (Bentley & Schmidt-Nielsen, 1966; Gunderson, Siegel & Leal, 2011). It is suggested, that large scales reduce the area of exposed, non-keratinized skin and thus reduce evaporative water loss (Alibardi, 2003). Accordingly, most studies exploring the adaptive significance of scale variation have focused on climatic correlates, particularly temperature and precipitation; most of these studies, however, have been limited to variation among populations or among a few closely related species, generally with mixed results (Bogert, 1949; Hellmich, 1951; Horton, 1972; Soulé & Kerfoot, 1972; Lister, 1976; Thorpe & Baez, 1987; 1993; Calsbeek, Knouft & Smith, 2006; Oufiero *et al*., 2011).

Anolis lizards are ideal candidates for studies of adaptive evolution—including variation in scalation—because of our detailed knowledge of their biology and evolutionary history (reviewed in Losos, 2009). Each island in the Greater Antilles (Cuba, Hispaniola, Jamaica, and Puerto Rico) contains a similar set of independently evolved microhabitat specialists or "ecomorphs"—species that are adapted to a specific niche (Williams, 1983; Losos, 2009). Ecomorphs differ in their use of the structural habitat (i.e., the structure of the vegetation they use, such as differences in height and diameter of perches) and exhibit corresponding differences in morphological traits related to structural habitat use such as body size, limb proportions, tail length, toepad size, and lamella number (specialized scales on the bottom of each digit that facilitate adhesion to smooth surfaces; for a review of the ecomorphs in *Anolis* see Losos, 2009). Biomechanical studies have shown that variation along these traits correlates with increased functional performance (Losos, 1990a; 1990b). The repeated evolution of particular ecomorphs on each island provides strong evidence that ecological processes may be responsible for generating functional and morphological diversity among species of anoles on these islands (Mahler *et al*., 2010).

Multiple species of the same ecomorph, however, occur on each of the four islands, often in sympatry. Additional axes of variation, including a physiological axis that encompass both thermal and hydric variation, explain how several species of otherwise similar *Anolis* can co-occur within similar microhabitats (e.g., Rodriguez Schettino *et al*., 2010; Hertz *et al*., 2013).

Scales in *Anolis* lizards vary interspecifically in size and shape and among body regions within individuals. To name a few features, dorsal scales can be granular, flat, keeled, smooth, circular, quadrangular, overlapping or with space between them. Smaller scales are generally granular and bigger scales tend to be flat. Ventral scales are flat, keeled or unkeeled, overlapping

or in close proximity (Figure 1). Scales on limbs, digits and the tail are flat, keeled and overlapping in most species.

In this paper, we examine variation of scale number in a broad, phylogenetically diverse group of *Anolis* lizards. Specifically, we test whether scale number correlates with a suite of climatic variables (the physiology hypothesis) across 142 species of *Anolis* lizards from the Lesser and Greater Antilles and the South and Central American Mainland. In addition, we test whether variation in scale size occurs along axes of structural microhabitat diversification (the ecomorph hypothesis).

Material and Methods

In most anole species, scale size is too small to be measured accurately; therefore, we used scale counts as an inverse measure of scale size (Smith, 1949; Oufiero *et al.,* 2011). Species can vary in the degree to which their skin is covered by scales, because of variation in the area of exposed skin between the scales. The relative contribution of scales and the interstitial skin to evaporative water loss or thermoregulation is, however, unknown. For the species used in this study, the relation between scales and the interstitial skin area was assessed and found to be in agreement with the assumption that scale number is a measure of scale size or scale coverage (see the supporting information for a detailed description).

Scale number was collected from both dorsal and ventral surfaces of 142 species of *Anolis* at the Harvard Museum of Comparative Zoology (MCZ). We measured both dorsal and ventral scales because they can differ in size within an individual. Only adult male individuals were used for this study. Males tend to be morphologically more differentiated than females and sexes differ in

aspects of the structural microhabitat (i.e. perch height) and are likely to exhibit different selection pressures (Losos 2009). Scales were counted by a single person (J.E.W.) along a one centimeter transect, from anterior to posterior, on dorsal and ventral body regions. Dorsal scales were counted parallel to the spine starting at shoulder level where the forelimb connects to the body. Ventral scales were counted parallel to the midbody, ending at level of the hindlimb. To account for body size, snout-vent length of each animal was measured with a ruler. When possible, multiple individuals were measured and an average scale count used in statistical analyses (supporting information, Table S2).

Phylogenetic information, including relationships among species and branch lengths, was taken from the time-calibrated molecular phylogeny of Mahler *et al*. (2010). Taxa in the tree, but not included in our dataset, were pruned. The final tree (Figure S3) had 142 species. To test for phylogenetic signal in the data, we estimated Blomberg's K using the 'phytools' package (Revell, 2012) in R (R Core Development Team, 2012). Any possible confounding effects of body size were removed using a phylogenetic regression (Revell, 2009) of scale number and snout-vent length (SVL). The phylogenetic size correction takes into account that species data are not independent due to shared evolutionary history. All measurements were log-transformed prior to the analyses. Statistical analyses were carried out in R (version 3.0.1; R Core Development Team 2012).

Different sets of explanatory variables were tested. First, we examined the relationship between scalation and climatic environment (the physiology hypothesis). We extracted data on 19 bioclimatic variables and altitude based upon the geographic coordinates of specimen localities. Climate data were downloaded from the WordClim database (www.worldclim.org; version 1.4; Hijmans *et al*., 2005). The bioclimatic variables represent monthly measures for

both precipitation and temperature at a 1-km^2 resolution. In addition, we calculated a single measure for aridity, the Q index:

$$\mathbf{Q} = \frac{\mathbf{Precipitation}}{(((\mathbf{Tmax} + \mathbf{Tmin})(\mathbf{Tmax} - \mathbf{Tmin}))\times\mathbf{1000})}$$

in which lower measures of Q indicate more arid environments (Oufiero *et al*., 2011). Species means were taken for each variable and a phylogenetic principal components analysis was performed to account for covariation among those variables. We used a phylogenetic generalized least square model (PGLS) to test for correlations between relative number of scales and bioclimatic variables, using the scores from the first three principal component axes (PC). For PC axes that showed significant correlations with scale number, we tested the bioclimatic variables individually correcting *P*-values for multiple testing with the Bonferroni method.

Second, we tested whether variation in scalation is associated with variation in the structural microhabitat (the ecomorph hypothesis). For this hypothesis, lizards were grouped into one of the six recognized ecomorph categories (Williams, 1983; Losos, 2009): trunk, trunk-ground, twig, grass-bush, crown-giant, and trunk-crown. We used perch height and perch diameter to account for specific aspects of the structural microhabitat. Species not assigned to an ecomorph category or for which no perch data were available were excluded from the analysis. The analysis was performed with the 'phytools' package (Revell, 2012) in R (R Core Development Team, 2012) using a phylogenetic ANOVA (*sensu* Blomberg, Garland & Ives, 2003) with post-hoc comparisons among groups using a sequential-Bonferroni method (Holm-Bonferroni). To test for correlations between scalation and continuous variables of the microhabitat (i.e. perch height and perch diameter), we used a phylogenetic linear square model (PGLS) of the 'caper' package (version 0.5; Orme *et al*., 2012) in R (R Core Development Team, 2012).

Results

Within a 1-centimeter transect, scale counts varied from five to 92 for dorsal scales and 11 to 53 in ventral scales. Phylogenetic signal was significant for all variables (Table1). Based on these results, the effect of body size was removed from the data with a phylogenetic regression using a Brownian motion model for evolution (Revell, 2009). The relationship between body size and scale number is shown in Figure S2, including estimates of intraspecific variation.

The hypothesis that variation in scale size correlates with variation in climatic environment is supported by our data. The first three axes of the principal component analysis account for 78.6% of the climatic variation (Table 2). Based on the loadings, the first principal component axis can be interpreted as a temperature and altitude axis, the second axis loads most strongly on precipitation variables and the third on temperature changes throughout the year. We found that dorsal and ventral scale number increase significantly with PC2, a measure for precipitation (dorsal: $P < 0.001$; $R^2 = 0.075$; ventral: $P = 0.001$; $R^2 = 0.066$; Table 3, Figure 2).

To untangle which individual precipitation variables correlate with relative number of scales, we tested the precipitation variables and the measure for aridity (index Q) separately. We found that dorsal scale counts correlate significantly with annual precipitation, dorsal and ventral scale counts correlate significantly with precipitation of the driest month, the driest quarter of the year and the coldest quarter, and ventral scale counts correlate negatively with precipitation seasonality (Table 4).

Variation in both dorsal and ventral scale counts differs among ecomorphs (dorsal: $P = 0.001$; $F = 10.68$; ventral: $P = 0.001$; $F = 5.87$; Table 5 for pairwise comparison). Grass-bush

ecomorphs have the largest body scales, after the effect of size was removed. Trunk-ground ecomorphs have the smallest dorsal scales and trunk ecomorphs the smallest ventral scales (Figure 3). Furthermore, scale number correlates significantly positively with perch height (N = 54 species; dorsal: $P = 0.034$; $R^2 = 0.081$; ventral: $P < 0.001$; $R^2 = 0.234$; Table 6, Figure 4) and perch diameter for dorsal scales (N = 54 species, $P = 0.005$; $R^2 = 0.136$; Table 6, Figure 4).

Discussion

Evolutionary diversification of *Anolis* lizards has become a textbook example of adaptive radiation. Most attention has focused on repeated patterns of adaptive diversification in traits such as limb length and toepad size to adapt to using different structural microhabitats, such as tree canopies, twigs, and grass (Losos, 2009). However, a second axis of evolutionary diversification has occurred as species occupying the same structural microhabitat have diverged to use different thermal microhabitats (Hertz *et al*., 2013).

Anoles exhibit extensive diversity in scale number, but this variation has not been considered in the context of anole macroevolutionary diversity. Consequently, in this study we quantified scale number across a large sample of species of the genus *Anolis* and examined whether this variation was related either to structural or climatic habitat diversity.

Scale number was found to vary with precipitation among 142 species that occur across a broad climatic range. Scales of species in wetter environments are more numerous (and thus smaller) compared with those in drier regions. This agrees with previous intraspecific studies in anoles (Lister, 1976; Calsbeek *et al*., 2006) and interspecific studies in other lizard taxa (e.g., *Sceloporus,* Oufiero *et al*., 2011), which show that populations in warmer and drier environments have fewer, larger scales than those in colder and wetter habitats. In contrast, Malhotra and

Thorpe (1997) found a negative relationship between scale number and precipitation in *Anolis oculatus*. To what degree intraspecific variation of scalation follows this pattern remains to be tested in future studies. Functional hypotheses that could explain these patterns have been raised in support of both outcomes (Losos, 2009): if water loss occurs through the scales, then scales should be smaller, reducing total evaporative surface area, in xeric regions. If, however, water evaporates mainly through the skin between scales, then the opposite might be expected. Our data are in agreement with most previous studies, showing that xeric species have larger scales, thereby suggesting that water loss through the interstitial skin is the key factor regulating scale size. Detailed physiological studies directly addressing this point are now needed to test this hypothesis (see also Kattan & Lillywhite, 1989 who showed that water loss through the skin decreases in *A. carolinensis* in xeric conditions).

Related to water loss, it is hypothesized that larger scales radiate more heat and thus could function as a heat shield more effectively than small scales (Soulé, 1966). Our results, similar to other broad interspecific studies in geographically widespread lizard taxa (Oufiero *et al*., 2011), did not support the hypothesis that larger scales are found in warmer environments.

Selection on scale size and number also may correlate with factors other than precipitation and temperature, such as protection from abrasion or signaling (e.g., light may reflect differently off of keeled and rugose scales than it does from a smooth and flat scale; Arnold, 2002). We found that relative scale size varies among the ecomorphs, which are adapted to use different structural microhabitats. In addition, particular aspects of the microhabitat (e.g. perch height and perch diameter) were found to correlate positively with scale number. Why more numerous (and thus smaller) scales would be advantageous on higher or broader perches is

not clear; this finding calls for further investigation of how scale size may relate functionally to differences in structural habitat.

Previous work has shown that the ecomorphs vary in traits such as limb length and toepad size, which are relevant for moving on different surfaces (reviewed in Losos, 2009). Why the size of body scales should vary with structural microhabitat is unclear. Members of the same ecomorph group can occur in very different climatic conditions and thus the physiology-hypothesis alone cannot explain this observation. Our findings suggest that other factors related to the ecomorph groups are involved in shaping scale characters. An alternative explanation, of course, is that scale characters are linked to other functional traits that are under selection and thus evolve in a hitchhiking fashion. Clearly, more work is needed to understand the putative adaptive basis of these convergent patterns of scale evolution.

The adaptive basis of anole evolution has been extensively studied. Our work supports previous suggestions of a relationship between scale size and hydric environment, with a sample size substantially greater than previous studies. In addition, our finding of a relationship with structural habitat is unexpected. Even after decades of work, much remains to be learned about the functional basis of anole diversification.

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Tables

Table 1: phylogenetic signal was significant for all variables using Blomberg's K. P-values <0.05 are indicated with an asterisk.

	K	*P*
SVL*	0.86	0.001
Number of dorsal scales*	0.66	0.001
Number of ventral scales*	0.61	0.001

Table 2: Loadings of a phylogenetic principal component analysis on nineteen bioclimatic variables and altitude, eigenvalues and variance in %. Only those axis with percentage variance greater than 10% were used for the analysis. The first principal component axis has high loadings on temperature variables and altitude. The second axis has high loading for precipitation variables and the third axis loads high on annual temperature variation.

Bioclimatic Variables	PC1	PC2	PC3
Annual Mean Temperature	0.977	0.148	0.044
Mean Diurnal Range	-0.398	-0.265	0.083
Isothermality	-0.118	0.211	-0.726
Temperature Seasonality	-0.077	-0.221	0.947
Max Temperature Warmest Month	0.916	0.029	0.242
Min Temperature Coldest Month	0.947	0.245	-0.166
Temperature Annual Range	-0.351	-0.385	0.629
Mean Temperature Wettest Quarter	0.931	0.027	0.200
Mean Temperature Driest Quarter	0.951	0.210	-0.106
Mean Temperature Warmest Quarter	0.964	0.107	0.211
Mean Temperature Coldest Quarter	0.955	0.190	-0.153
Annual Precipitation	-0.410	0.862	0.060
Precipitation Wettest Month	-0.433	0.693	0.002
Precipitation Driest Month	-0.126	0.826	0.163
Precipitation Seasonality	-0.171	-0.686	-0.183
Precipitation Wettest Quarter	-0.459	0.714	0.036
Precipitation Driest Quarter	-0.137	0.853	0.170
Precipitation Warmest Quarter	-0.550	0.481	0.239
Precipitation Coldest Quarter	0.021	0.847	0.048
Altitude	-0.932	-0.199	-0.190
Eigenvalue	8.407	5.090	2.224
Percentage variance	42.033	25.452	11.121

Table 3: Multivariate phylogenetic linear regression (PGLS) shows interaction between relative scale number (residuals from a phylogenetic regression of scale number and SVL) and bioclimatic variables represented as three principal component axes. PC2, a measure for precipitation (Table 1) correlates significantly with scale number of dorsal and ventral scales (indicated with asterisk).

	Estimate	SE	t	*P*
		Dorsal		
PC1	<0.001	0.001	-0.334	0.739
PC2*	0.003	0.001	3.638	<0.001
PC3	<0.001	0.001	0.321	0.748
		Ventral		
PC1	<0.001	<0.001	0.360	0.719
PC2*	0.002	<0.001	3.420	0.001
PC3	<0.001	0.001	0.591	0.556

Table 4: Univariate phylogenetic linear regression (PGLS) shows interaction between relative scale number (residuals from a phylogenetic regression of scale number and SVL) and individual precipitation variables and a measure for aridity (index Q). This analysis was based on results from previous multivariate analysis (significant correlation of PC2 with relative number of dorsal and ventral scales; Table 3) to untangle, which individual precipitation variables are predictors for scale number. Asterisks indicate significant correlations after correcting *P*-values for multiple testing (Bonferroni method).

	Estimate	SE	t	*P*	Corrected *P*	r^2
			Dorsal			
Annual Precipitation*	<0.001	<0.001	2.935	0.004	0.035	0.058
Precipitation Wettest Month	<0.001	<0.001	1.267	0.207	1.000	0.011
Precipitation Driest Month*	0.001	<0.001	3.074	0.003	0.022	0.063
Precipitation Seasonality	-0.002	0.001	-2.414	0.017	0.153	0.040
Precipitation Wettest Quarter	<0.001	<0.001	2.156	0.033	0.294	0.032
Precipitation Driest Quarter*	<0.001	<0.001	2.993	0.003	0.029	0.060
Precipitation Warmest Quarter	<0.001	<0.001	1.568	0.119	1.000	0.017
Precipitation Coldest Quarter*	<0.001	<0.001	3.465	0.001	0.006	0.078
Aridity Index Q	16.798	8.875	1.893	0.060	0.543	0.025
			Ventral			
Annual Precipitation	<0.001	<0.001	2.482	0.014	0.127	0.042
Precipitation Wettest Month	<0.001	<0.001	1.318	0.190	1.000	0.012
Precipitation Driest Month*	0.001	<0.001	3.041	0.003	0.025	0.062
Precipitation Seasonality*	-0.001	<0.001	-3.137	0.002	0.018	0.065
Precipitation Wettest Quarter	<0.001	<0.001	1.478	0.142	1.000	0.015
Precipitation Driest Quarter*	<0.001	<0.001	3.043	0.003	0.025	0.062
Precipitation Warmest Quarter	<0.001	<0.001	0.964	0.337	1.000	0.007
Precipitation Coldest Quarter*	<0.001	<0.001	3.079	0.002	0.022	0.063
Aridity Index Q	9.045	5.280	1.713	0.089	0.800	0.020

Table 5: *P*-values of a pairwise comparison (phylogenetic ANOVA) among ecomorph groups and relative number of dorsal and ventral scales (residuals from a phylogenetic regression of scale number and SVL).

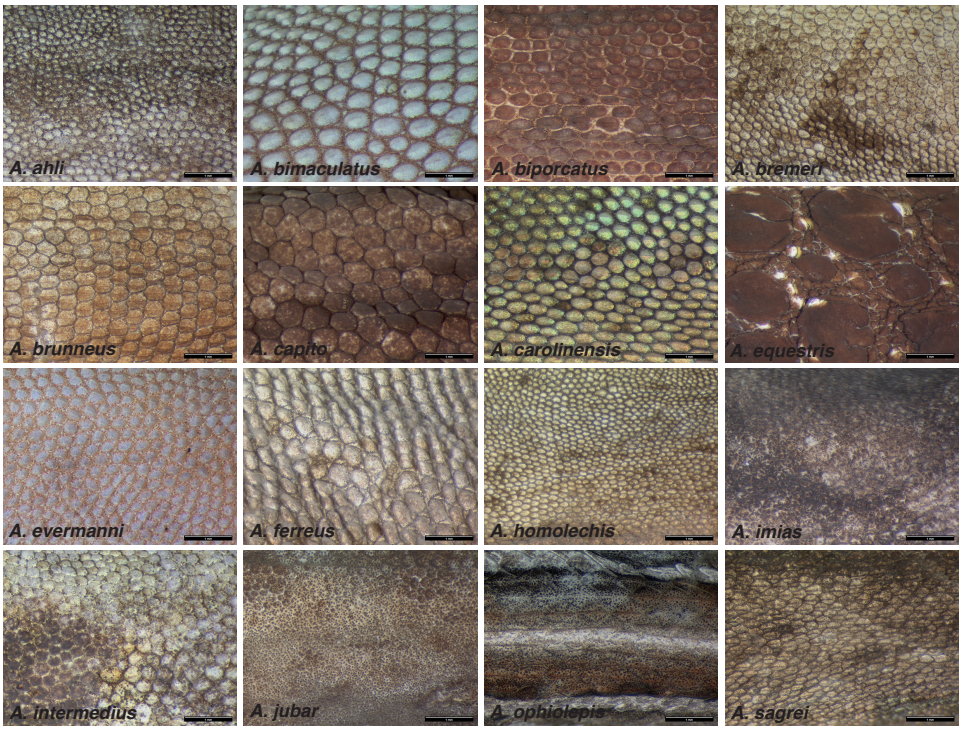
	Crown-Giant		Grass-Bush		Trunk		Trunk-Crown		Trunk-Ground		Twig	
	P	t	*P*	t	*P*	t	*P*	t	*P*	t	*P*	t
						Dorsal						
Crown-Giant	1.000	0.000	1.000	0.707	0.020	-3.002	0.048	-2.526	0.015	-4.670	0.045	-2.966
Grass-Bush	1.000	-0.707	1.000	0.000	0.015	-3.715	0.015	-3.628	0.015	-6.308	0.015	-3.823
Trunk	0.020	3.002	0.015	3.715	1.000	0.000	1.000	1.240	1.000	-0.059	1.000	0.373
Trunk-Crown	0.048	2.526	0.015	3.628	1.000	-1.240	1.000	0.000	0.119	-2.135	1.000	-0.948
Trunk-Ground	0.015	4.670	0.015	6.308	1.000	0.059	0.119	2.135	1.000	0.000	1.000	0.595
Twig	0.045	2.966	0.015	3.823	1.000	-0.373	1.000	0.948	1.000	-0.595	1.000	0.000
						Ventral						
Crown-Giant	1.000	0.000	0.026	2.873	1.000	0.464	1.000	-0.084	0.380	1.869	0.380	-2.055
Grass-Bush	0.026	-2.873	1.000	0.000	0.528	-1.673	0.026	-3.281	0.528	-1.454	0.015	-4.735
Trunk	1.000	-0.464	0.528	1.673	1.000	0.000	1.000	-0.552	1.000	0.841	0.380	-2.110
Trunk-Crown	1.000	0.084	0.026	3.281	1.000	0.552	1.000	0.000	0.198	2.222	0.380	-2.120
Trunk-Ground	0.380	-1.869	0.528	1.454	1.000	-0.841	0.198	-2.222	1.000	<0.001	0.015	-3.965
Twig	0.380	2.055	0.015	4.735	0.380	2.110	0.380	2.120	0.015	3.965	1.000	0.000

Table 6: Univariate phylogenetic regression (PGLS) shows interaction between relative scale number (residuals from a phylogenetic regression of scale number and SVL), perch height and perch diameter. Significant *P*-values (≤ 0.05) are indicated with an asterisk.

	Estimate	SE	t-value	p-value	r^2
Dorsal					
Perch diameter*	0.050	0.017	2.901	0.005	0.136
Perch height*	0.078	0.036	2.175	0.034	0.081
Ventral					
Perch diameter	0.018	0.009	1.956	0.056	0.067
Perch height*	0.068	0.017	4.025	<0.001	0.234

Figure 1: Photographs of dorsal (A) and ventral (B) scales of selected specimens. Scales of *Anolis* lizards vary in size and shape. Dorsal scales can be granular or flat, keeled or unkeeled. Larger scales tend to be flat while smaller scales are granular. Ventral scales are mostly flat, keeled or smooth and can overlap. Within an individual, dorsal and ventral scales can differ in size and shape. Ventral scales can be smaller or larger than dorsal scales. Black bars = 1mm.

A


A. ahli
A. bimaculatus
A. biporcatus
A. bremeri
A. brunneus
A. capito
A. carolinensis
A. equestris
A. evermanni
A. ferreus
A. homolechis
A. imias
A. intermedius
A. jubar
A. ophiolepis
A. sagrei

B

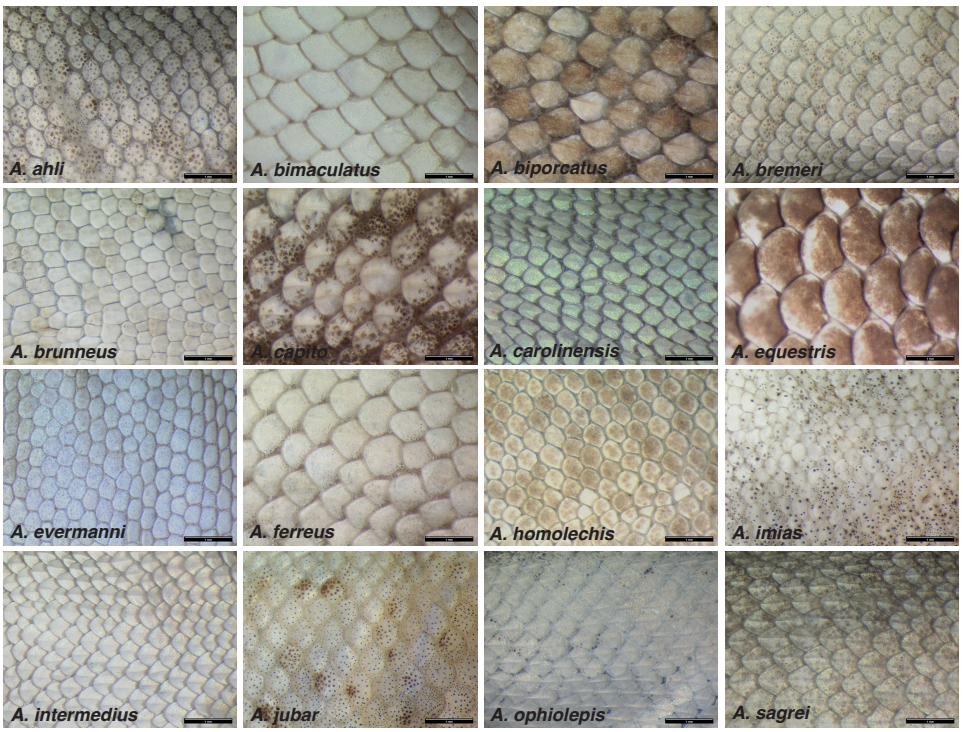

A. ahli
A. bimaculatus
A. biporcatus
A. bremeri
A. brunneus
A. capito
A. carolinensis
A. equestris
A. evermanni
A. ferreus
A. homolechis
A. imias
A. intermedius
A. jubar
A. ophiolepis
A. sagrei

Figure 2: Relative number of dorsal (A) and ventral (B) scales (residuals from a phylogenetic regression of scale number and SVL) of 142 lizard species correlates significantly with measures of precipitation (dorsal: $P < 0.001$; ventral: $P = 0.001$; for PC2 loadings see Table 1). The regression lines do not represent the statiscical phylogenetic linear model used for the analysis (results shown in Table 2). Black circles represent species means.

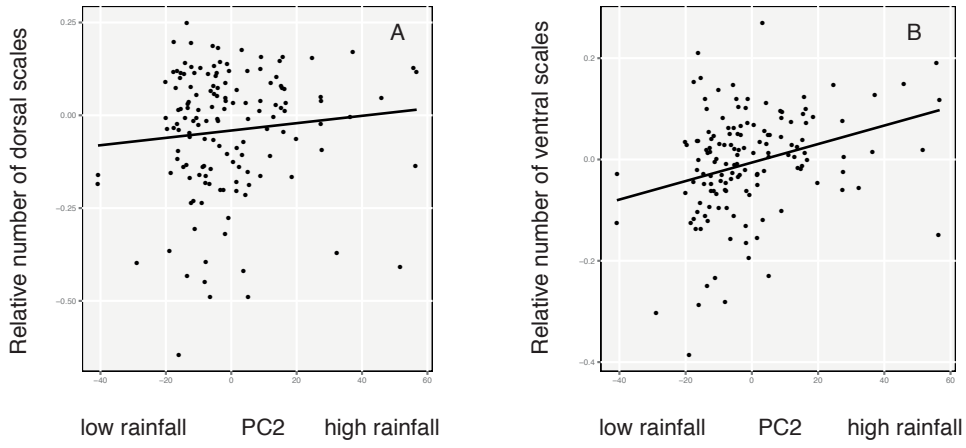


Figure 3: Group means of ecomorphs and relative number of dorsal and ventral scales with standard deviations (bars). Ecomorph groups differ significantly in number of ventral and dorsal scales (dorsal: $P = 0.001$; ventral: $P = 0.001$; phylogenetic ANOVA). Mainland species were excluded from the statistical analysis because they could not be assigned to ecomorph groups, but are shown in the Figure (gray circles).

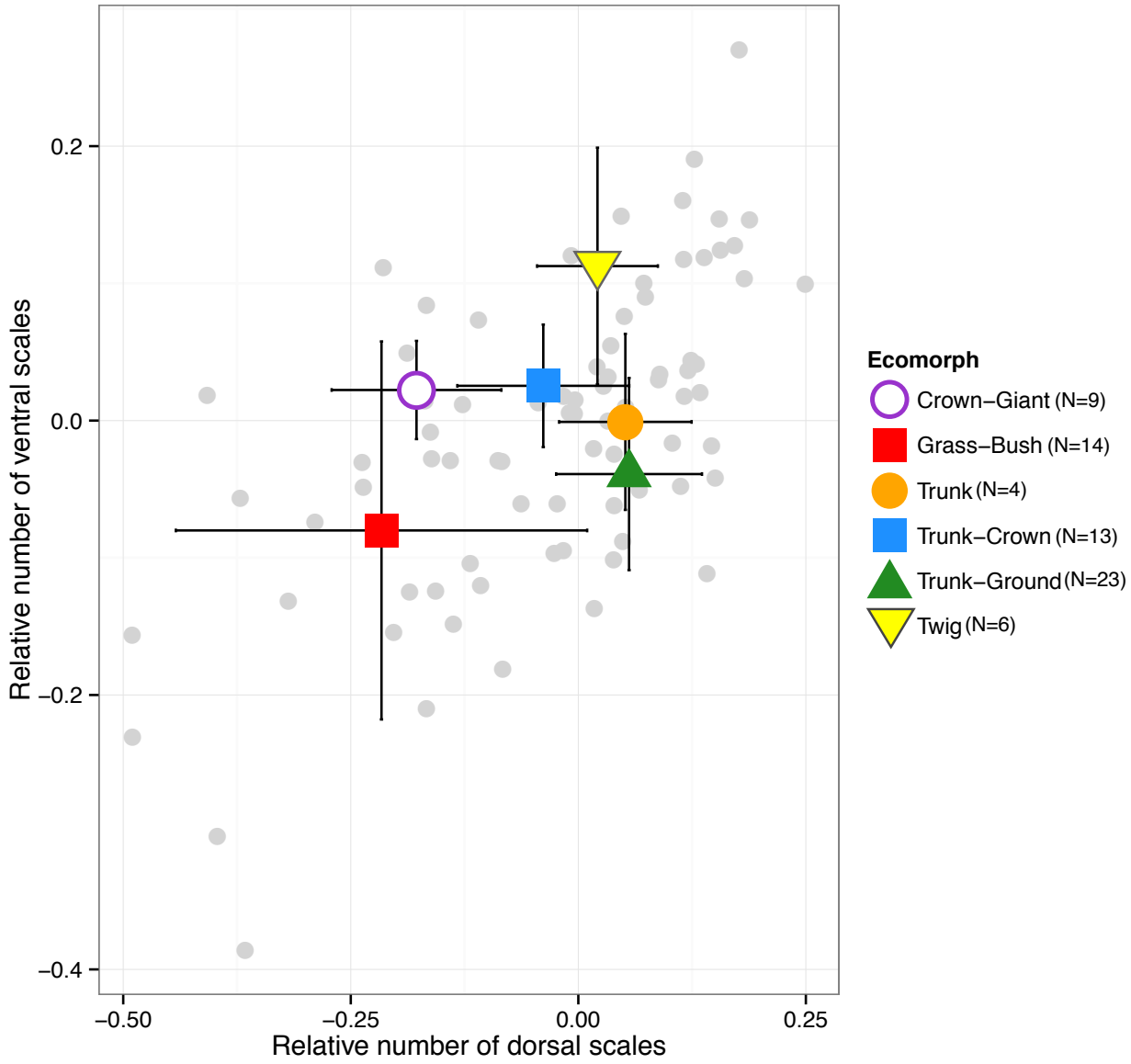


Figure 4: Relative number of scales (residuals from a phylogenetic regression of scale number and SVL) of 54 lizard species correlates with perch height (A and B) and perch diameter (C and D). Black circles represent species means. The regression lines do not represent the phylogenetic linear model (PGLS) used for the analysis (Table 5). Correlations of dorsal scale number are significant for perch height ($P = 0.034$) and perch diameter ($P = 0.005$). Number of ventral scales correlates significantly with perch height ($P < 0.001$).